Kidney International, Vol. 23 (1983), pp. 401–406

Extra-renal production of 24,25-dihydroxyvitamin D in chronic renal failure during 25 hydroxyvitamin D_3 therapy

JOSEPH E. ZERWEKH, JOHN J. MCPHAUL, JR, TOM F. PARKER, and CHARLES Y. C. PAK

The Mineral Metabolism Section, Department of Internal Medicine, Southwestern Medical School, and the Medical Service, Dallas Veterans Administration Medical Center, Dallas, Texas

Extra-renal production of 24,25-dihydroxyvitamin D in chronic renal failure during 25 hydroxyvitamin D_3 therapy. To assess whether or not production of 24,25-dihydroxyvitamin D [24,25-$(OH)_2D$] can occur in patients with renal failure, we administered 25-OHD_3 (100 μg/day) to eight patients undergoing chronic hemodialysis, six of whom had intact kidneys (CRF) and two who were anephric. Prior to 25-OHD_3 administration, serum 24,25-$(OH)_2D$ was undetectable (UD), (<0.3 ng/ml) in all subjects except for one patient with CRF and one anephric subject in whom concentrations of 24,25-$(OH)_2D$ were 0.5 ng/ml and 1.5 ng/ml, respectively. The administration of 25-OHD_3 (100 μg/day) for 8 weeks to six CRF and two anephric patients produced significant increases in serum 24,25-$(OH)_2D$ concentration, from UD to 2.9 ± 0.5 SEM ng/ml ($P < 0.0025$) in CRF, and from 0.9 ± 0.4 ng/ml to 2.6 ± 0.8 ng/ml ($P < 0.005$) in the anephric group. Serum 24,25-$(OH)_2D$ concentration was found to be correlated significantly with serum 25-OHD concentration ($r = 0.93$, $P < 0.01$). Serum 1,25-$(OH)_2D$ did not change in anephric subjects but significantly increased in patients with CRF (9 ± 3 to 14 ± 3 pg/ml, $P < 0.05$). Additional studies in seven normal subjects and seven anephric patients who received 1,25-$(OH)_2D$ (2 μg/day) for 8 days disclosed a significant increase in serum 24,25-$(OH)_2D$ in normals (2.4 ± 0.2 ng/ml to 3.3 ± 0.3 ng/ml, $P < 0.05$) but not in the anephric humans. The data suggest that there is an impaired ability to bioproduce 24,25-$(OH)_2D$ in anephric humans or in CRF, but that sufficient provision of the substrate (25-OHD_3) may result in the extra-renal production of 24,25-$(OH)_2D$. The extra-renal production of 24,25-$(OH)_2D$ does not appear to be regulated in a comparable fashion to that of the renal enzyme, since 1,25-$(OH)_2D$ administration failed to raise 24,25-$(OH)_2D$ concentration in anephric subjects. Despite low serum 1,25-$(OH)_2D$ concentrations in patients with CRF, 25-OHD_3 therapy may also raise 1,25-$(OH)_2D$ levels in some patients, suggesting the presence of residual 1α-hydroxylase activity.

La production extra-rénale de 24,25-dihydroxyvitamine dans l'insuffisance rénale chronique pendant un traitement par la 25-hydroxyvitamine D_3. Afin de déterminer si la production de 24,25-dihydroxyvitamine D [24,25-$(OH)_2D$] peut avoir lieu chez des malades en insuffisance rénale chronique, nous avons administré de la 25-OHD_3 (100 μg/jour) à huit malades en hémodialyse chronique, dont six avaient des reins intacts (CRF) et deux étaient anéphriques. Avant l'administration de 25-OHD_3, la 24,25-$(OH)_2D$ sérique était indétectable (UD), (<0,3 ng/ml) chez tous les sujets sauf un malade CRF et un sujet anéphrique chez lesquels les concentrations de 24,25-$(OH)_2D$ étaient de 0,5 ng/ml et de 1,5 ng/ml, respectivement. L'administration de 25-OHD_3 (100 μg/jour) pendant 6 semaines à six malades CRF et deux anéphriques a entraîné des augmentations significatives de la concentration sérique de 24,25-$(OH)_2D$, passant de UD à 2,9 ± 0,5 SEM ng/ml ($P < 0,0025$) chez les CRF et de 0,9 ± 0,4 ng/ml à 2,6 ± 0,8 ng/ml ($P < 0,005$) chez le groupe anéphrique. La concentration sérique de 24,25-$(OH)_2D$ a été trouvée significativement corrélée à la concentration sérique de 25-OHD ($r = 0,93$, $P < 0,01$). La 1,25-$(OH)_2D$ sérique n'a pas changé chez les sujets anéphriques, mais s'est significativement élevée chez les malades CRF (de 9 ± 3 à 14 ± 3 pg/ml, $P < 0,05$). Des études supplémentaires chez sept sujets normaux et sept malades anéphriques ayant reçu de la 1,25-$(OH)_2D$ (2 μg/jour) pendant huit jours ont révélé une augmentation significative de la 24,25-$(OH)_2D$ sérique chez les normaux (2,4 ± 0,2 ng/ml à 3,3 ± 0,3 ng/ml, $P < 0,05$) mais pas chez les anéphriques. Ces données suggèrent qy'il y a une capacité altérée à produire de la 24,25-$(OH)_2D$ chez des hommes anéphriques ou CRF, mais qu'un apport suffisant du substrat (25-OHD_3) peut entraîner une production extra-rénale de 24,25-$(OH)_2D$. La production extra-rénale de 24,25-$(OH)_2D$ ne parait pas soumise à la même régulation que l'enzyme rénale, puisque l'administration de 1,25-$(OH)_2D$ n'a pas permis d'augmenter la concentration de 24,25-$(OH)_2D$ chez des sujets anéphriques. Malgré de faibles concentrations de 1,25-$(OH)_2D$ chez les malades avec CRF, le traitement par la 25-OHD_3 peut également élever le niveau de 1,25-$(OH)_2D$ chez certains malades, suggérant la présence d'une activité 1α-hydroxylase résiduelle.

The kidney has been shown to be an important site of vitamin D metabolism. Bioproduction of 1,25-dihydroxyvitamin D [1,25-$(OH)_2D$], the physiologically active form of vitamin D, is known to occur principally in renal mitochondria [1] and is regulated tightly by the calcium and phosphorus status [2, 3]. Another renal metabolite of vitamin D whose bioproduction appears to be regulated is 24,25-dihydroxyvitamin D [24,25-$(OH)_2D$] [4]. The activities of the 25-hydroxyvitamin D (25-OHD) 1α- and 24-hydroxylase, the enzymes responsible for the production of 1α,25-$(OH)_2D$ and 24,25-$(OH)_2D$, respectively, have been reported to be reciprocal. Under conditions of vitamin D depletion 25-OHD is preferentially metabolized to 1,25-$(OH)_2D$ but during vitamin D replete states 24,25-$(OH)_2D$ is bioproduced. Thus, the renal 24-hydroxylase is stimulated by 1,25-$(OH)_2D$ or normal serum calcium but suppressed by parathyroid hormone (PTH). These reciprocal changes in enzyme activity have been demonstrated both in vivo [4, 5] and in vitro [6, 7].

Although reports have demonstrated the existence of extra-renal sites of 1,25-$(OH)_2D$ production in placenta [8, 9] and bone cells [10], still to be resolved is whether or not 24,25-$(OH)_2D$ is exclusively a product of the kidney. Recent studies

Received for publication December 15, 1981
and in revised form August 5, 1982

0085–2538/83/0023–0401 $01.20

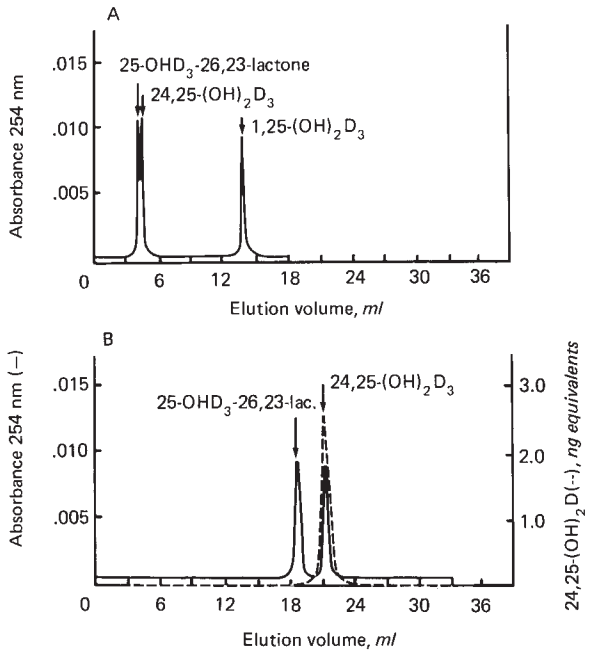

Fig. 1. **A** *Elution of 25-OHD$_3$-26,23-lactone, 24,25-(OH)$_2$D$_3$, and 1,25-(OH)$_2$D$_3$ from an HPLC Supelcosil column developed in 8:92 isopropanol:hexane with a flow rate of 1.0 ml/min.* **B** *Elution of 25-OHD$_3$-26,23-lactone and 24,25-(OH)$_2$D$_3$ from an HPLC Supelcosil column developed in 3:97 isopropanol: hexane with a flow rate of 1.0 ml/min.* The *solid line* represents the elution profile of standards while the *dotted line* represents the elution of material found to compete in the 24,25-(OH)$_2$D assay. This material was obtained from an anephric serum pool during 25-OHD$_3$ therapy.

have demonstrated low [11] to normal [12, 13] concentrations of 24,25-(OH)$_2$D in anephric human serum, presumably a reflection of extra-renal 25-OHD 24-hydroxylase activity [14, 15]. In contrast to these reports, Taylor et al [16] and Taylor [17] failed to detect 24,25-(OH)$_2$D in serum of anephric man with normal or above normal 25-OHD concentrations. These results have been confirmed recently by Horst et al [18]. However, studies in anephric pigs [18], rats [19], and most recently humans [20] have demonstrated extra-renal production of 24,25-(OH)$_2$D$_3$ when serum 25-OHD$_3$ concentration is elevated by administration of this vitamin D metabolite or its parent form.

To address the issue of whether or not extra-renal production of 24,25-(OH)$_2$D can occur in severe renal failure, we have measured the serum concentrations of 24,25-(OH)$_2$D, 25-OHD, and 1,25-(OH)$_2$D in anephric patients and patients with chronic renal failure with intact kidneys (CRF) undergoing dialysis after 8 weeks of oral 25-OHD$_3$ therapy (100 μg/day). Seven additional anephric subjects and seven normal volunteers received 1,25-(OH)$_2$D$_3$ to determine whether extra-renal production of 24,25-(OH)$_2$D is regulated in a manner similar to the renal enzyme. This information could be of importance in the treatment and prevention of renal osteodystrophy since 24,25-(OH)$_2$D has been implicated in bone formation and mineralization [21, 22].

Methods

Sterols and assays. 25-OHD$_3$ and 1α,25-(OH)$_2$D$_3$ were gifts from the Upjohn Company, Kalamazoo, Michigan. 24,25-(OH)$_2$D$_3$ was a gift from Hoffmann-LaRoche, Nutley, New Jersey. 25-OHD$_3$-26,23 lactone was generously provided by Dr. J. Napoli, Department of Biochemistry, University of Texas, Southwestern Medical School, Dallas, Texas. All radioactive vitamin D metabolites used in the respective assays and for column chromatography standardization were purchased from Amersham-Searle, Arlington Heights, Illinois.

The procedure for the extraction of the vitamin D metabolites was identical to that described by Bishop et al [23]. The dried extract was then applied to a 1 × 10 cm column of Sephadex LH-20 (Sigma Chemical Co., St. Louis, Missouri) suspended in a chloroform-hexane (60:40) solvent system and developed in the same solvent system. The initial 3 ml were discarded and the next 6 ml collected as 25-OHD. The next 30 ml contained both 24,25-(OH)$_2$D and 1,25-(OH)$_2$D. Final separation and purification of the vitamin D metabolites were performed on a Glenco high pressure liquid chromatograph (Glenco Instruments, Houston, Texas) utilizing a Supelcosil 150 × 4.5 mm column (Supelco, Bellefonte, Pennsylvania). 25-OHD was purified with a 5% 2-propanol in hexane solvent system. 24,25-(OH)$_2$D was separated from 1,25-(OH)$_2$D, and final purification of both metabolites was accomplished with an 8% 2-propanol in hexane solvent system. Final yields for each metabolite averaged 60%.

Serum 25-OHD and 24,25-(OH)$_2$D were assayed separately utilizing the rat serum binding protein system [24]. The sensitivity of each assay is 0.3 ng/ml of serum when using a 2-ml serum sample and performing assays in triplicate.

Our described purification scheme for 24,25-(OH)$_2$D used for each patient's serum in this study does not resolve this metabolite from 25-OHD$_3$-26,23 lactone, (Fig. 1**A**) a vitamin D metabolite that can compete approximately five times better than 24,25-(OH)$_2$D in the binding assay [18]. However, a 3% 2-propanol in hexane solvent system was found to effectively separate 25-OHD$_3$-26,23 lactone from 24,25-(OH)$_2$D$_3$. Utilizing this solvent system, we failed to observe any measurable quantity of 25-OHD$_3$-26,23 lactone in a pooled 5-ml serum sample from either the two anephric subjects or two CRF patients after 8 weeks of 25-OHD$_3$ therapy (Fig. 1**B**). However, significant concentrations of 24,25-(OH)$_2$D were present in these pooled serums suggesting that only 24,25-(OH)$_2$D was being bioproduced in each individual after 25-OHD$_3$ administration and not 25-OHD$_3$-26,23 lactone.

Assay of sera for 1,25-(OH)$_2$D was performed in triplicate on each sample as previously described [25] except that separation of free sterol from bound was accomplished with hydroxylapatite [26]. The limit of detection with this assay utilizing radioactive 1,25-(OH)$_2$D at a specific activity of 110 Ci/mmole is 8 pg/ml of serum when using a 2-ml serum sample with 60% recovery during purification. No attempt was made to determine the concentration of the vitamin D$_2$ forms of each metabolite. Since our chromatographic procedures were standardized to collect only the D$_3$ form of the vitamin, we do not know if the D$_2$ form of each metabolite was also being measured. Thus, the values obtained in this study represent total vitamin D concentrations and are thus designated without a subscript.

Serum immunoreactive parathyroid hormone (iPTH) was determined using antiserum (Diagnostic Systems Laboratories, Webster, Texas) which recognized principally the carboxyterminus to the PTH molecule. Bovine PTH was used as the tracer, and human PTH (from culture of human parathyroid tissue

Table 1. Basal vitamin D metabolite concentrations in chronic hemodialysis patients[a]

Group	25-OHD *ng/ml*	$24,25\text{-}(OH)_2D$[d] *ng/ml*	$1,25\text{-}(OH)_2D$[e] *pg/ml*
Normal range (25)	24 ± 3	2.5 ± 0.4	34 ± 2
CRF; intact kidneys (6)	16 ± 2[b]	0.3 ± 0.1[b]	9 ± 3[c]
Anephric (9)	14 ± 1[b]	0.4 ± 0.3[b]	8 ± 1[c]

[a] Numbers in parentheses represent the number of patients in each group. All values are mean ± SEM.
Values were significantly different from the normal mean value at: [b] $P < 0.005$ and [c] $P < 0.001$.
[d] When $24,25\text{-}(OH)_2D$ values were undetectable, the limit of sensitivity of the assay (0.3 ng) was used for calculation purposes.
[e] When $1,25\text{-}(OH)_2D$ values were undetectable, the limit of sensitivity of the assay (8 pg) was used for calculation purposes.

Table 2. Effect of $25\text{-}OHD_3$ administration in chronic hemodialysis patients

Group	Treatment	25-OHD *ng/ml*	$24,25\text{-}(OH)_2D$[a] *ng/ml*	$1,25\text{-}(OH)_2D$[b] *pg/ml*	iPTH *μlEq/ml*	Serum Ca *mg/dl*	Serum P *mg/dl*
Normal subjects ($N = 25$)	—	24 – 3	2.5 ± 0.4	34 ± 2	< 36	8.5 – 10.5	2.5 – 4.5
CRF, intact kidneys ($N = 6$)	Basal	16 ± 2	0.3 ± 0.1	9 ± 3	182 ± 39	9.2 ± 0.3	3.3 ± 0.7
	$25\text{-}OHD_3$ (100 μg/d for 8 weeks)	98 ± 10[e]	2.9 ± 0.5[f]	14 ± 3[c]	188 ± 68	9.8 ± 0.3[c]	4.5 ± 0.5
Anephric patients ($N = 2$)	Basal	12 ± 3	0.9 ± 0.4	8 ± 1	296 ± 45	7.6 ± 0.4	4.2 ± 0.8
	$25\text{-}OHD_3$ (100 μg/d for 8 weeks)	82 ± 28[e]	2.6 ± 0.8[e]	8 ± 1	254 ± 31	8.1 ± 0.1	7.4 ± 2.9[d]

[a] When $24,25\text{-}(OH)_2D$ values were undetectable, the limit of sensitivity of the assay (0.3 ng) was used for calculation purposes.
[b] When $1,25\text{-}(OH)_2D$ values were undetectable, the limit of sensitivity of the assay (8 pg) was used for calculation purposes.
Values were significantly different from the basal value as calculated by paired t test at
[c] $P < 0.05$;
[d] $P < 0.025$;
[e] $P < 0.005$;
[f] $P < 0.0025$.

kindly provided by Dr. B. Roos, Case Western Reserve University, Cleveland, Ohio) was used as the standard. This assay provided detectable values in more than 95% of normal subjects tested as previously demonstrated [27]. Although this assay demonstrated elevated iPTH values for all patients with CRF, this may not be a true measure of biologically active hormone concentration. This is based upon observations which demonstrate the predominance of C-region iPTH fragments (presumed to be biologically inert) relative to intact PTH in CRF serum.

Study protocol. Six patients with stable chronic renal failure (CRF) (creatinine clearance <2 ml/min) who were undergoing hemodialysis (HD) and two anephric subjects undergoing HD also underwent oral $25\text{-}OHD_3$ therapy (100 μg/day) for a period of 8 weeks. Patients were selected randomly from those who had not received any vitamin D supplementation. Serum was obtained at 2-week intervals for measurement of vitamin D metabolites, iPTH, serum calcium and phosphorus.

Seven normal subjects and seven additional anephric subjects were assessed prior to and following 8 days of oral $1,25\text{-}(OH)_2D_3$ therapy (2 μg/day in four divided doses). Serum was obtained for analysis as described above. All patients were on their usual diets during the study period, and none had been treated with vitamin D prior to the study. Results are expressed as the mean ± SEM. Significance was determined by use of Student's t test for between group comparisons and the paired t test for within group comparisons.

Results

Basal vitamin D metabolite concentrations. Prior to the initiation of $25\text{-}OHD_3$ administration, no $24,25\text{-}(OH)_2D$ was detected in the serum of eight of nine anephric subjects or in five of six patients with CRF. Values of 1.5 and 0.5 ng/ml were disclosed in an anephric subject and in one patient with CRF, respectively. Although serum 25-OHD levels were significantly lower than the normal mean concentration, the values were within the normal range (16 ± 2 SEM ng/ml) in CRF and for the nine anephric subjects (14 ± 1 ng/ml) (Table 1). As expected, serum $1,25\text{-}(OH)_2D$ was reduced significantly for patients with CRF (9 ± 3 pg/ml, $P < 0.001$) and for the anephric subjects (8 ± 1 pg/ml, $P < 0.001$) as compared to the normal value of 34 ± 2 pg/ml.

Effect of $25\text{-}OHD_3$ administration in anephric humans and in CRF. Administration of $25\text{-}OHD_3$ (100 μg/day) for 8 weeks resulted in a marked elevation of serum 25-OHD concentration in both anephric subjects and for the six patients with CRF (Table 2). By 8 weeks 25-OHD concentrations reached a mean

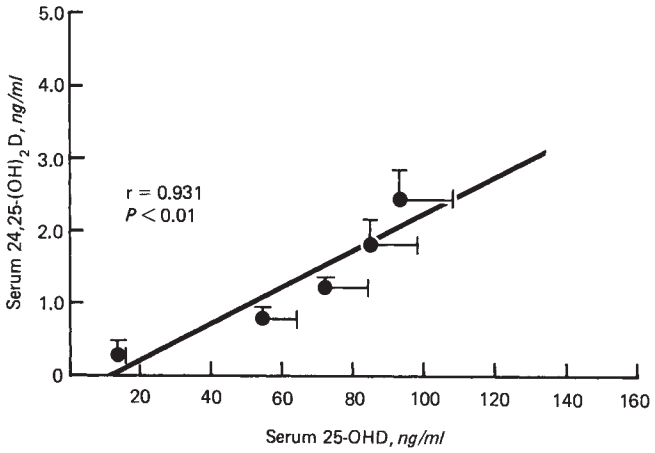

Fig. 2. *Correlation of serum 24,25-$(OH)_2D$ concentration with serum 25-OHD concentration during an 8-week administration of 25-OHD_3 (100 μg/d) in six hemodialysis patients with CRF and intact kidneys (N = 6) and two anephric subjects on chronic hemodialysis (N = 2).* Each *point* corresponds to the basal value and reevaluation at 2, 4, 6, and 8 weeks of 25-OHD_3 administration. Values shown are mean ± SEM for all eight subjects. Regression analysis yielded a significant correlation ($P < 0.01$).

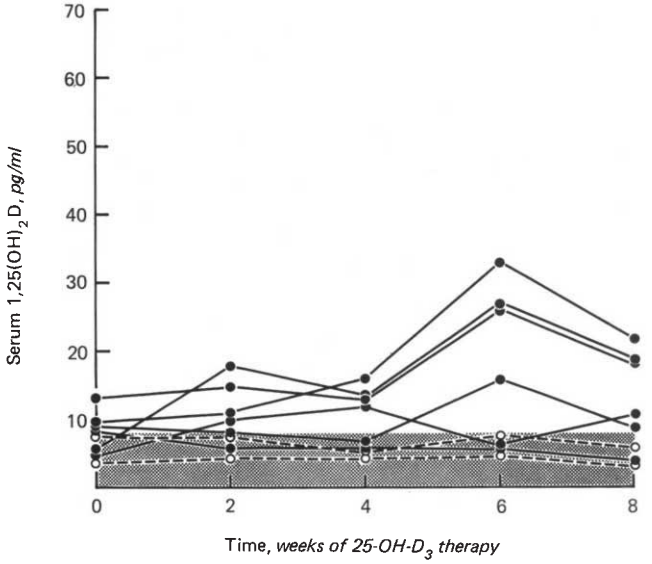

Fig. 3. *Serum concentration of 1,25-$(OH)_2D$ in six hemodialysis subjects with CRF and intact kidneys and two anephric subjects on hemodialysis who received 25-OHD_3 for 8 weeks (100 μg/d).* Each *line* represents a study in a single patient and is depicted by CRF (●—●) and anephrics (○—○). The limit of sensitivity for the assay is represented by the *shaded region.*

value of 98 ± 10 ng/ml in CRF and 82 ± 28 ng/ml for the two anephric subjects. Both mean values were significantly different ($P < 0.005$) from the pre-treatment serum concentration of 25-OHD. An increase in the serum concentration of 24,25-$(OH)_2D$ occurred, commensurate with the rise in serum 25-OHD. This was true for patients with CRF (mean value, 2.9 ± 0.5 ng/ml, $P < 0.0025$ from pre-treatment) and for anephric subjects (mean value, 2.6 ± 0.8, $P < 0.005$ from pre-treatment; Table 2). Furthermore, the increase in serum 24,25-$(OH)_2D$ concentration was correlated positively with the increase in serum 25-OHD concentration for the entire 8-week period ($r = 0.93$, $P < 0.01$; Fig. 2).

Although administration of 25-OHD_3 failed to raise serum 1,25-$(OH)_2D$ levels in anephric patients, a significant increase did occur for patients with CRF and intact kidneys (9 ± 3 to 14 ± 3 pg/ml, $P < 0.05$; Table 2). Three CRF patients demonstrated serum 1,25-$(OH)_2D$ concentrations within the normal range at 6 weeks of therapy (Fig. 3), but the remaining three CRF subjects showed no significant elevation in 1,25-$(OH)_2D$.

During 25-OHD_3 administration there were no significant changes in iPTH or serum P in chronic HD patients with intact kidneys. The two anephric subjects demonstrated a fall in iPTH and a significant increase in serum phosphorous (Table 2). Serum calcium was increased significantly ($P < 0.025$) by 8 weeks of 25-OHD_3 administration in HD patients with CRF and intact kidneys. Hypercalcemia was evident in two of these latter CRF subjects at 8 weeks of 25-OHD_3 therapy. Discontinuance of the drug corrected the hypercalcemia.

Effect of 1,25-$(OH)_2D_3$ administration in normal and anephric humans. The results of 1,25-$(OH)_2D_3$ administration in normal and anephric humans is summarized in Table 3. Two micrograms per day of 1,25-$(OH)_2D_3$ produced a significant increase in the serum concentration of 1,25-$(OH)_2D$ in both groups. Serum 25-OHD levels remained unchanged while the mean serum 24,25-$(OH)_2D$ concentration significantly increased in normal subjects (2.4 ± 0.2 ng/ml to 3.3 ± 0.3, $P < 0.05$). Seven anephric subjects failed to demonstrate an increase in the serum concentration of 24,25-$(OH)_2D$, and all values remained undetectable.

Serum iPTH decreased in both patient groups during 1,25-$(OH)_2D_3$ administration. This change was significant for the anephric subjects (Table 3). There were no significant changes in serum calcium or phosphorus.

Discussion

Our results have demonstrated very low to nondetectable levels of 24,25-$(OH)_2D$ in serum from anephric patients undergoing hemodialysis as well as HD patients with intact kidneys. This finding agrees with previous reports supporting a lack of measurable 24,25-$(OH)_2D$ in serum of anephric humans [16–18]. One subject with CRF and one anephric patient had relatively normal levels of 24,25-$(OH)_2D$. Because our assay does not discriminate between the D_2 and D_3 forms of the vitamin, we were unable to determine if these normal values were due to prior vitamin D_2 therapy.

The major purpose of this study was to assess whether patients with renal failure on dialysis with or without intact kidneys have the capacity to produce 24,25-$(OH)_2D$. In anephric people extra-renal tissues such as the intestine [14] or bone could be such a site of production [15]. When superphysiological serum 25-OHD concentrations were achieved by exogenous 25-OHD_3, a significant (and qualitatively similar) increase in the serum concentration of 24,25-$(OH)_2D$ was found in all six patients with CRF and in both anephric subjects. The mean increase in serum 24,25-$(OH)_2D$ concentration correlated positively with the mean increase in serum 25-OHD concentration.

These data are in contrast to the findings of Taylor et al [16] and Taylor [17] who found undetectable values for 24,25-

Table 3. Effect of $1,25\text{-}(OH)_2D_3$ administration in normal and anephric humans

Group	Treatment	25-OHD *ng/ml*	$24,25\text{-}(OH)_2D$[a] *ng/ml*	$1,25\text{-}(OH)_2D$[b] *pg/ml*	iPTH *μlEq/ml*	Serum Ca *mg/dl*	Serum P *mg/dl*
Normals ($N = 7$)	Basal	34 ± 6	2.4 ± 0.2	26 ± 4	30 ± 2	9.5 ± 0.1	3.3 ± 0.3
	$1,25\text{-}(OH)_2D_3$ (2 μg/d for 8 days)	32 ± 6	3.3 ± 0.3[c]	46 ± 5[d]	28 ± 2	9.5 ± 0.1	3.8 ± 0.4
Anephric patients ($N = 7$)	Basal	14 ± 3	0.3 ± 0.1	8 ± 1	254 ± 19	9.4 ± 0.3	5.9 ± 0.5
	$1,25\text{-}(OH)_2D_3$ (2 μg/d for 8 days)	13 ± 2	0.3 ± 0.1	89 ± 15[e]	226 ± 31[c]	9.8 ± 0.4	6.4 ± 0.5

[a] When $24,25\text{-}(OH)_2D$ values were undetectable, the limit of sensitivity of the assay (0.3 ng) was used for calculation purposes.
[b] When $1,25\text{-}(OH)_2D$ values were undetectable, the limit of sensitivity of the assay (8 pg) was used for calculation purposes.
Significantly different from the basal value as calculated by paired *t* test at
[c] $P < 0.05$.
[d] $P < 0.005$.
[e] $P < 0.001$.

$(OH)_2D$ in anephric subjects who had been dosed with vitamin D_3 or $25\text{-}OHD_3$. This discrepancy might be explained by the relatively short (4 days) period of $25\text{-}OHD_3$ administration in the study of Taylor et al [16]. Using a comparable dose of $25\text{-}OHD_3$ (100 μg/day), we observed that not all patients demonstrated increases in serum $24,25\text{-}(OH)_2D$ by 2 weeks of therapy. In fact, 4 weeks or longer of daily $25\text{-}OHD_3$ administration was required for all patients to achieve increased serum $24,25\text{-}(OH)_2D$ concentrations. This observation may indicate that the extra-renal 25-OHD-24-hydroxylase may have a higher K_m than that for the renal enzyme.

Alternatively, our isolation and purification scheme for $24,25\text{-}(OH)_2D$ does not separate $25\text{-}OHD_3\text{-}26,23$ lactone from $24,25\text{-}(OH)_2D$. Since this metabolite has been shown to compete with $24,25\text{-}(OH)_2D$ in the rat serum binding protein assay [18], the possiblity is raised that our assay may be detecting this metabolite also. This is unlikely since production of $25\text{-}OHD_3\text{-}26,23$ lactone requires intact renal function [28, 29]. Furthermore, we were unable to detect any $25\text{-}OHD_3\text{-}26,23$ lactone in the serum from CRF patients and anephric subjects after 8 weeks of $25\text{-}OHD_3$ administration when the putative $25\text{-}OHD_3\text{-}26,23$ lactone peak was separated by high pressure liquid chromatography using a 3% 2-propanol in hexane solvent system, despite measureable levels of $24,25\text{-}(OH)_2D$. No attempts were made to determine if $25,26\text{-}(OH)_2D$, another metabolite which can compete in the $24,25\text{-}(OH)_2D$ assay [11], was present in the serum of CRF and anephric subjects before and following $25\text{-}OHD_3$ administration. Although previous studies have demonstrated a lack of $25,26\text{-}(OH)_2D_2$ in anephric serum [18], others have demonstrated the capacity of anephrics to bioproduce $25,26\text{-}(OH)_2D$ [11, 13]. However, our purification step with high pressure liquid chromatography should resolve $24,25\text{-}(OH)_2D$ from $25,26\text{-}(OH)_2D$ as shown by others [30] using a similar solvent system. Thus, it would appear that the metabolite in question is indeed $24,25\text{-}(OH)_2D$.

An additional finding in this study was the apparent ability of some HD patients with intact kidneys and CRF to bioproduce $1,25\text{-}(OH)_2D$ when sufficient substrate ($25\text{-}OHD_3$) is provided. It is not known whether this 1α-hydroxylation is of renal or extra-renal origin or a combination of both. Although a previous report suggests the presence of extra-renal 1α-hydroxylase activity in humans [20], our anephric subjects had undetectable values of $1,25\text{-}(OH)_2D$ in the basal state and during $25\text{-}OHD_3$ administration and does not support a role for extra-renal 1α-hydroxylase activity under these conditions. Studies in additional anephric subjects will be required to address this issue. These observations would also suggest that residual renal 24-hydroxylase activity might be present in patients with CRF with intact kidneys and that the increase in serum $24,25\text{-}(OH)_2D$ concentration observed in this study might be due to the renal enzyme. However, the magnitude of increase in serum $24,25\text{-}(OH)_2D$ for patients with CRF as compared to the anephric subjects was comparable, indicating similar 24-hydroxylase activities. Furthermore, the elevated iPTH levels observed in these patients would promote the stimulation of renal 1-hydroxylase activity and not 24-hydroxylase. These considerations support the notion that the observed increases in serum $24,25\text{-}(OH)_2D$ in CRF subjects after $25\text{-}OHD_3$ administration are a result of extra-renal 24-hydroxylation.

The nature and site of this hypothesized extra-renal 24-hydroxylation is unknown. It does not appear to be identical to the renal enzyme with regard to regulation since we failed to detect an increase in serum $24,25\text{-}(OH)_2D$ in anephric subjects who received $1,25\text{-}(OH)_2D$. This regimen was shown to significantly increase $24,25\text{-}(OH)_2D$ in normal subjects in this study and by others [17]. Although comparable studies in CRF patients were not performed in this investigation, similar findings to those observed in anephrics would provide further support for the hypothesis that the extra-renal enzyme is not regulated tightly. Furthermore, it still remains to be determined whether or not raising serum $24,25\text{-}(OH)_2D$ concentrations in anephric patients by giving exogenous $25\text{-}OHD_3$ offers any distinct advantage to $1,25\text{-}(OH)_2D_3$ in the management of renal osteodystrophy.

Acknowledgments

The study was supported by grants R01-AM16061, P50-AM20543, M01-RR00633 from the National Institutes of Health and by a grant from the Upjohn Company, Kalamazoo, Michigan. The authors thank E. Faris for assistance.

Reprint requests to Dr. J. E. Zerwekh, Mineral Metabolism Section, Department of Internal Medicine, Southwestern Medical School, 5323 Harry Hines Boulevard, Dallas, Texas 75235, USA

References

1. Fraser DR, Kodicek E: Unique biosynthesis by kidney of a biologically active vitamin D metabolite. *Nature (Lond)* 228:764–766, 1970
2. DeLuca HF, Schones HK: Metabolism and mechanism of action of vitamin D. *Annu Rev Biochem* 45:631–666, 1976
3. Haussler MR, McCain TA: Basic and clinical concepts related to vitamin D metabolism and action. *N Engl J Med* 297:974–983, 1041–1050, 1977
4. Tanaka Y, Lorenc RS, DeLuca HF: The role of 1,25-dihydroxyvitamin D_3 and parathyroid hormone in the regulation of chick renal 25-hydroxy-vitamin D_3-24-hydroxylase. *Arch Biochem Biophys* 171:521–525, 1975
5. Henry HL: Regulation of the hydroxylation of 25-hydroxyvitamin D_3 in vivo and in primary cultures of chick kidney cells. *J Biol Chem* 254:2722–2729, 1979
6. Kawashima H, Torikai S, Kurokawa K: Localization of 25-hydroxyvitamin D_3 1α-hydroxylase and 24-hydroxylase along with the rat nephron. *Proc Natl Acad Sci USA* 78:1199–1203, 1981
7. Juan D, DeLuca HF: The regulation of 24,25-dihydroxyvitamin D_3 production in cultures of monkey kidney cells. *Endocrinology* 101:1184–1193, 1977
8. Tanaka HF, Halloran B, Schones HK, DeLuca HF: The in vitro production of 1,25-dihydroxyvitamin D_3 by rat placental tissue. *Proc Natl Acad Sci USA* 76:5033–5035, 1979
9. Whitsett JA, Ho M, Tsang RC, Norman EJ, Adams KG: Synthesis of 1,25-dihydroxyvitamin D_3 by human placenta in vitro. *J Clin Endocrinol Metab* 53:484–488, 1981
10. Turner RT, Puzas JE, Forte MD, Lester GE, Gray TK, Howard GA, Baylink DJ: In vitro synthesis of 1α,25-dihydroxycholecalciferol and 24,25-dihydroxycholecalfciferol by isolated calvarial cells. *Proc Natl Acad Sci USA* 77:5720–5724, 1980
11. Horst RL, Shepard RM, Jorgensen NA, DeLuca HF: The determination of 24,25-dihydroxyvitamin D and 25,26-dihydroxyvitamin D in plasma from normal and nephrectomized man. *J Lab Clin Med* 93:277–285, 1979
12. Haddad JG Jr, Min C, Mendelsohn M, Slatopolsky E, Hahn T: Competitive protein-binding radioassay of 24,25-dihydroxyvitamin D in sera from normal and anephric subjects. *Arch Biochem Biophys* 182:390–395, 1977
13. Shepard RM, Horst RL, Hamstra AJ, DeLuca HF: Determination of vitamin D and its metabolites in plasma from normal and anephric man. *Biochem J* 182:55–69, 1979
14. Kumar R, Schnoes HK, DeLuca HF: Rat intestinal 25-hydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_3-24-hydroxylase. *J Biol Chem* 253:3804–3809, 1978
15. Garabedian M, Corvol MR, Bailly du Bois M, Liberherr M, Balsan S: The activity of 24,25-dihydroxycholecalciferol on cultured chondrocytes and its in vitro production in cartilage and calvarium, in *Endocrinology of Calcium Metabolism*, edited by Copp DH, Talmadge RV, Amsterdam, Excerpta Medica, 1977, p. 372
16. Taylor CM, Mawer CB, Wallace JB, St. John J, Cochran M, Russell RGG, Kanis JA: The absence of 24,25-dihydroxycholecalciferol in anephric patients. *Clin Sci* 55:541–547, 1978
17. Taylor CM: 24,25-Dihydroxyvitamin D in human serum, in *Vitamin D: Basic Research and its Clinical Application*, edited by Norman AW, Schaefer K, Herrath DV, Grigoleit H-G, Coburn JW, DeLuca HF, Mawer EB, Suda T, Berlin, Walter De Gruyter, 1979, pp. 197–203
18. Horst RL, Littledike ET, Gray RW, Napoli JL: Impaired 24,25-dihydroxyvitamin D production in anephric human and pig. *J Clin Invest* 67:274–280, 1981
19. Garabedian M, Pavolovitch H, Fellot C, Balsan S: Metabolism of 25-hydroxyvitamin D_3 in anephric rats: A new active metabolite. *Proc Natl Acad Sci USA* 71:534–557, 1974
20. Lambert PW, Stern PH, Avioli RC, Brockett NC, Turner RT, Greene A, Fu IY, Bell NH: Evidence for extrarenal production of 1α,25-dihydroxyvitamin D in man. *J Clin Invest* 69:722–725, 1982
21. Bordier D, Rasmussen H, Marie P, Miravet L, Gueris J, Ryckwaert A: Vitamin D metabolites and bone mineralization in man. *J Clin Endocrinol Metab* 46:284–294, 1978
22. Fournier A, Bordier P, Gueris J, Sebert JL, Marie P, Ferriere C, Bedrossian J, DeLuca HF: Comparison of 1α-hydroxycholecalciferol and 25-hydroxycholecalciferol in the treatment of renal osteodystrophy: Greater effect of 25-hydroxycholecalciferol on bone mineralization. *Kidney Int* 15:196–204, 1979
23. Bishop JE, Norman AW, Coburn AW, Roberts PA, Henry HL: Studies on the metabolism of calciferol XVI: Determination of the concentration of 25-hydroxyvitamin D, 24,25-dihydroxyvitamin D and 1,25-dihydroxyvitamin D in a single two-milliliter plasma sample. *Miner Electrolyte Metabol* 3:181–189, 1980
24. Haddad JG, Chyu KF: Competitive protein binding assay for 25-hydroxycholecalciferol. *J Clin Endocrinol Metab* 33:992–995, 1971
25. Zerwekh JE, Sanders LA, Towensend J, Pak CYC: Tumoral calcinosis: Evidence for concurrent defects in renal tubular phosphorus transport and in 1α,25-dihydroxycholecalciferol synthesis. *Calcif Tissue Int* 32:1–6, 1980
26. Wecksler WR, Norman AW: An hydroxylapatite batch assay for the quantitation of 1α,25-dihydroxyvitamin D_3-receptor complexes. *Anal Biochem* 92:314–323, 1979
27. Pak CYC, Nicar MJ, Peterson R, Zerwekh JE, Snyder W: A lack of unique pathophysiological background for nephrolithiasis of primary hyperparathyroidism. *J Clin Endocrinol Metab* 553:536–547, 1981
28. Horst RL, Littledike ET: 25-OHD_3-26,23 lactone: Demonstration of its kidney dependent synthesis. *Biochem Biophys Res Commun* 93:149–154, 1980
29. Tanaka Y, Wichmann JK, Paaren HE, Schnoes HK, DeLuca HF: Role of kidney tissue in the production of 25-hydroxyvitamin D_3-26,23-lactone and 1α,25-dihydroxyvitamin D_3-26,23-lactone. *Proc Natl Acad Sci USA* 77:6411–6414, 1980
30. Napoli JL, Okita RT, Masters BS, Horst RL: Identification of 25,26-dihydroxyvitamin D_3 as a rat renal 25-hydroxyvitamin D_3 metabolite. *Biochemistry* 20:5865–5871, 1981